(free) end to a microscopic bead. The DNA tether restricts the Brownian motion of the bead and the average amplitude of excursions of the bead from the anchor point indicates the DNA tether length. Excursions of the beads depend on the physical properties of the DNA-bead system, and the physical and chemical properties of the surrounding solution. Previously published TPM calibrations, for a range of DNA lengths and 200 to 970 nm diameter beads showed that larger beads increase excursions and comparatively more time is necessary for a bead to fully explore the available volume and report the maximum excursion for a longer as opposed to a shorter DNA molecule. Others have shown that magnesium ions reduce the excursions and increased viscosity slows the diffusion of an 80 nm gold bead tethered by a single length of DNA. New experiments with 320 and 480 nm diameter beads and DNA of several lengths show increasing viscosity increases the time required to report the maximum average excursion. Interpreting these results based on the dynamics of the viscous solvent-DNA-bead system highlights important experimental details for TPM measurements of DNA protein interactions and indicates how the kinetics of topological rearrangements might be impacted by increased viscosity environments such as cytoplasm.

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Application of 3-D Prismatic Optical Tracking to Single-Molecule Optical Tweezers
Takayuki Nishizaka, Tatsuya Naito, Yuh Hasimoto, Tomoko Masaike.
Gakushuin Univ., Tokyo, Japan.
We have developed a new method for tracking single particles in three dimensions under a conventional microscope by dividing light in two components using a wedge prism. This method, three-dimensional prismatic optical tracking, termed ***tPOT***, is here combined with optical tweezers to quantify molecular scale 3-D force in motor proteins. Precise calibration of trapping force to bead displacement with sub nanometer scale enables us to determine the force exerted not only in *xy*-plane direction but also along optical axis (*z*-axis). With this experimental set-up, we applied force to rotary motor F_1-ATPase that showed rotation on a glass surface. Our research group previously used the procedure in which the objective was displaced to impose the load on single molecules held between the trapped bead and the glass, which frequently caused the measurement error because of drifting motions of the specimen. This method has been modified as follows; 1) the trap center is displaced with time by moving the focusing lens along *z*-axis; 2) spring constant of the trap is increased via the adjustment of the laser power. In either case positions of the stage and the objective were fixed and thus repetitive measurements were reliably performed. The rupture force of the bead from the glass surface, which presumably reflects the interaction between the shaft and the cylinder of F_1-ATPase, distributed up to 40 pN and showed 19 pN on the average. The wide distribution of the force may be originated from different chemical states of the cylinder when the rupture occurs. We also demonstrate various applications of ***tPOT***, such as corkscrewing motions of linear molecular motors or beating motions of ciliary axonemes.

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Molecular Mechanotransduction in Human CD4
Raul Perez Jimenez, Ronen Berkovich, Patricia Richard, Carmen Badilla, Julio Fernandez.
COLUMBIA UNIVERSITY, New York, NY, USA.
HIV-1 infection initiates when the viral envelope glycoprotein gp120 interacts with two receptors on the surface of the T lymphocytes, CD4 and a chemokine co-receptor (CCR5 or CXCR4). Upon binding, a cascade of conformational changes in CD4 triggers viral fusion to the cell membrane. However, the key factors that originate the structural alterations in CD4 are unclear. Here, we use single-molecule force spectroscopy to study the mechanochemical properties of the two more external domains of human CD4 (D1 and D2). The application of mechanical forces to the CD4 domains reveals that they can be unfolded in a time-dependent manner within a biological range of forces. Similarly to other tandem repeats proteins such as titin or tenascin, mechanical unfolding of CD4 modules might be an important process occurring in vivo acting as a shock absorber. This phenomenon would help to prevent viral detachment. Furthermore, we show that mechanical exposition of internal disulfide bonds in CD4 is required for redox regulation by thioredoxin enzymes, a process that has been suggested to occur during viral infection. In addition, we perform numerical calculations using suitable models for polymer elasticity to correlate viral infectivity (Freeman et al. Structure 18(12):163241) with mechanical extensibility of CD4 modules. The role of mechanical forces in HIV-1 infection is yet to be considered, but it might represent a new view to better understand viral infection.

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Single-Molecule Optical Trap Study of Human CCR5 mRNA Structure
Michel de Messieres, Jen-Chien Chang, Ashton T. Belew, Arturas Meskauskas, Jonathan D. Dinman, Arthur La Porta.
University of Maryland, College Park, MD, USA.
Tertiary structure of mRNA can regulate translation by the ribosome. Specifically, potential pseudoknot structures are found in many genes which may stimulate programmed −1 ribosomal frame shifting. We conducted single-molecule optical trapping experiments on CCR5 mRNA, which encodes the co-receptor for HIV-1. We found that individual molecules of CCR5 mRNA will randomly fold into several unique conformations when stretched repeatedly, one of these being consistent with a proposed pseudoknot structure. Other conformations have higher energies than would be expected for duplex RNA and may be explained by tertiary RNA structure. A new analysis technique was applied, where we consider the change in energy per distance (dE/dx) of the molecule. This method of analysis provides a useful means to classify the many conformations the mRNA adopts as well as providing unique insights into the structure of CCR5 mRNA.

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Fluorogen Activating Peptides that Enhance Photostability through Encapsulation of the Fluorogen
Saumya Saurabh, Marcel P. Bruchez.
Carnegie Mellon University, Pittsburgh, PA, USA.
Biological imaging has profited from the development of a variety of fluorescent probes such as organic dyes, quantum dots and fluorescent proteins. More recently, Fluorogen Activating Peptides (FAP) have been developed through directed evolution. These FAPs bind non-covalently to an otherwise non-fluorescent molecule (fluorogen) to form fluorescent complexes. FAPs have been successfully employed in various live-cell imaging applications in the past two years. For live-cell and single molecule imaging applications, photostability is a key requirement for any fluorescent probe. FAPs have been shown to exhibit high photostability in cell-imaging applications. An understanding of the mechanism underlying the photostability of these FAPs will help us in future to develop FAP clones with higher photostability. In this study, we have characterized the photobleaching behavior of two Malachite green (MG) binding FAP clones: dL5NP138 and HL4. These studies have been performed at a single molecule level. These studies have shown FAPs to be more photostable compared to a widely used single molecule dye, Cy5, at a single molecule condition. Additionally we have exposed FAPs and the Green fluorescent protein to vigorous oxidizing conditions to assess the encapsulation of the chromophore in the protein environment.
Our studies show that FAPs protect MG (the fluorogen) from external environments that are rich in reactive oxygen species. The same behavior was observed by GFP compared to a synthetic soluble GFP chromophore. Using single molecule approaches in the presence of excess dye, we have demonstrated that these MG-binding FAPs are photostable through encapsulation of the fluorogen, and not by exchange of the bleached fluorogen. This mechanism provides MG-FAP complexs that can be used for single molecule detection over periods greater than Cy5 labels, yet with the convenience of genetically encoded reporters such as GFP.

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Single Molecule Counting using STORM/PALM
Ulrich Schmidt, Jeffrey L. Werbin, Peter K. Sorger, Gaudenz Danuser.
Harvard Medical School, Boston, MA, USA.
Single-molecule counting using STORM/PALM
During the last decade, several super-resolution microscopy techniques have been developed that enable the circumvention or even the breaking of Abbe's diffraction limit. One approach of achieving super-resolution is to control the emission state of individual fluorophores in time and the concomitant ability to localize active emitters with up to nanometer accuracy. Two of the most commonly implemented super-resolution techniques are stochastic optical reconstruction microscopy (STORM) and photo-activated localization microscopy (PALM). Each technique uses the localization of individual emitters to improve resolution as has been demonstrated with numerous intracellular structures. Beyond that, these techniques open the possibility of counting on a single molecule level. However, the ability to count is fundamentally limited by the fluorophores used for these techniques which blink many times before photobleaching. A typical STORM dye like Cy5 can cycle between tens and hundreds of times between a dark and a fluorescent state before